BACTERIOLOGICAL, PHYSIOLOGICAL, ETC.

Methods of Bacterial Analysis of Air. G. L. A. Ruehle. (*J. Agric. Research*, 1915, **4**, 343-366.)—The author has made a critical investigation of the methods employed for estimating the bacterial contamination of air. Methods based on two general principles have been employed: (1) passing a known volume of air through a filtering apparatus (aeroscope) and counting the colonies developed from the filtering medium by plate-cultivation; (2) estimating the bacterial precipitation from the air on a known area in a definite time. Most of the work

recorded in the paper relates to the comparative efficiencies of various forms of aeroscope. Two types have been in general use hitherto; viz., the standard aeroscope recommended by the committee of the American Public Health Association, in which a filtering layer of sand is used, and Rettger's aeroscope, in which the air is bubbled through a small quantity of physiological salt solution. The author has adopted a slightly modified form of the standard sand-filter aeroscope which avoids certain small drawbacks of the original. It consists of a wide glass tube 70 mm. long and 15 mm. in diameter, fused to a narrow tube 40 mm. long and 6 mm. in diameter, by which it is attached to an aspirator. The upper end of the wide tube is closed by a cork through which passes a narrow tube of the same dimensions as the lower one, but bent at an angle of 45° to prevent the entrance of bacteria by gravitational precipitation. The lower portion of the wide tube is packed with a plug of cotton wool above which is a layer, 10 mm. thick, of sand which has passed through a 100-mesh sieve but been retained on a 160-mesh sieve. A measured volume of air is aspirated through the aeroscope at the rate of about 0·5 to 1·0 litre per minute; the sand is then shaken out into 10 c.c. of sterile water and aliquot portions of this suspension are cultivated on agar-agar plates. It was found that the standard form of aeroscope varied in its filtering efficiency from 50 to 100 per cent., with an average efficiency in two series of tests of 90 and 91·6 per cent. It is believed that the chief cause of error in this pattern arises from the fact that it is so constructed that it must be sterilised by steam, which causes caking in the sand filter. The modified pattern described above can be sterilised by dry heat, and was found to retain nearly 100 per cent. of the bacteria with little chance of error. For field work it is more convenient in every way than the liquid type of aeroscope recommended by Rettger, although this latter may be made to yield excellent results if manipulated with sufficient care. The method of determining bacterial precipitation from air by means of exposed Petri dishes containing solid nutrient is obviously quite unreliable, since it affords a measure only of the number of infected dust particles falling on a given area, irrespective of the number of bacteria conveyed by them. The latter figure is more correctly estimated by exposing sterile water in sterile vessels for a given time and inoculating plates from these. J. F. B.

Electrolytic Determination of Biological Solution. E. Pantanelli. (*Centralbl. für Bakt.*, 1914, **42**, 439-443; through *Bull. Agric. Intell. and Plant Diseases*, 1915, **6**, 663.)—In determining the part played by microbiological action in rendering the soil constituents available, some investigators have measured directly the electrolytic conductivity of water-saturated soil; the author modified this method by estimating the conductivity of the percolating solutions of the soil. He used in his experiments different soil samples from the neighbourhoods of Tripoli and of Naples. Through these he allowed: (*a*) sterilised water, (*b*) sterilised water saturated either with chloroform, or 0·5 per cent. dextrose, or (*c*), water mixed with dextrose and chloroform to percolate three times. He found that the determination of the electrolytic conductivity was a good method of estimating the microbiological solubility of the soil particles, especially if the experiment was carried out comparatively with and without the addition of chloroform and dextrose. Chloroform

increases, while dextrose decreases, but not always, the leaching out of the salts from the soil. The solubility of the soil usually varies with the number of micro-organisms it contains.

Reaction for Indole: Detection of Cholera Bacilli. O. Baudisch. (*Zeitsch. physiol. Chem.*, 1915, **94**, 132-135; through *J. Soc. Chem. Ind.*, 1915, **34**, 849.)—In testing for cholera bacilli a surface culture of the suspected organisms is made on agar, and after eight to sixteen hours a group of colonies is transferred with the supporting agar to a test-tube and heated to boiling with dilute potassium hydroxide solution containing a small quantity of nitromethane. After the solution has cooled somewhat it is shaken with 1 c.c. of amyl alcohol, and again after addition of an excess of strong hydrochloric acid. The alcoholic layer, which separates best if the liquid is warmed nearly to boiling, is coloured red or pink if the colonies have produced the slightest trace of indole. The nitromethane, instead of being added with the alkali, may be mixed with the agar before the latter has set, a few drops sufficing for a large quantity of the medium.

Papain: Its Commercial Preparation and Digestive Properties. D. S. Pratt. (*Philippine J. Sci.*, 1915, **10**, 1-33.)—Ceylon papain, prepared by drying the latex of the fruit, is dark brown to nearly black in colour, owing apparently to the presence of an oxydase; lighter grades are grossly adulterated generally by the addition of boiled rice starch; occasionally the dark and light grades are blended in the same sample. Mexican papain, more carefully prepared, is lighter brown in colour, and the best West Indian products resemble dried bread-crumbs. For the valuation of the proteolytic activity, skimmed milk is the best substratum; the author uses a 40 per cent. solution of sweetened condensed separated milk. The papain gum is not completely soluble in water: 0·75 grm. of powdered papain is digested with 150 c.c. of water at 40° C. for thirty minutes, and the solution filtered. For the standard test, 25 c.c. of milk solution and 23 c.c. of water are mixed with 2 c.c. of filtered papain solution, equivalent to 10 mgrms. of the dry sample, and the mixture is digested for thirty minutes at 40° C. At the end of that time, 20 c.c. of ice-water are added, and the flask is immediately cooled in an ice bath. The liquid is transferred to a beaker, and the undigested protein precipitated by slowly adding 0·5 c.c. of copper sulphate solution (60 grms. per litre), followed by 0·5 c.c. of glacial acetic acid with vigorous stirring. The contents of the beaker are placed in a 100 c.c. measuring cylinder, allowed to settle, and filtered through an ashless filter-paper 11 cm. in diameter, previously dried at 100° C. and tared. The curd is broken up with a rod, washed with water at 60° C., then dried at 100° C. and weighed. The protein digested is calculated by comparison with a blank experiment, and the proteolytic activity is expressed in terms of protein digested per unit weight of the sample on an average of six determinations; duplicate results should agree within about 2 per cent. The following results for activity numbers are recorded: (1) Commercial specimens, Ceylon, 0·1, 5·6, 9·6, 5·7, 22·0, 1·8; Mexico, 12·9; West Indies, 40·0. (2) Prepared in laboratory, Philippine fresh latex (calculated on dry substance) 45·8; ditto, sun-dried, 45·4; precipitated by alcohol, 72·2. With the weaker pre-

parations an increase in the quantity of papain employed increases the activity number; but with stronger specimens an increase in the quantity produces a very marked decrease in the activity per unit weight. The digestion of milk protein with papain at 40° C. proceeds rapidly during the first ten minutes, and practically reaches its maximum within an hour under the conditions specified. The addition of hydrochloric acid up to 0·06 per cent. has only a slight retarding influence on the digestion, but quantities from 0·06 to 0·13 per cent. very greatly reduce the activity of the enzyme, while a further increase up to 0·20 per cent. has practically no further effect. Sodium bicarbonate and sodium chloride in small proportions have no influence on the digestion. Hydrocyanic acid exerts a marked stimulating effect. Papain has a slight activity at 0° C., and at 70° C. its action in presence of large amounts of the enzyme is not greatly weakened. J. F. B.

Estimation of Pepsin. L. J. Geselschap. (*Zeitsch. physiol. Chem.*, 1915, **94**, 193-226; through *J. Soc. Chem. Ind.*, 1915, **34**, 848.)—The author investigated various methods for the estimation of pepsin in gastric juice. Those of Volhard and Fuld and Levison were found least satisfactory. Sufficiently accurate results can be obtained by Mett's method, which is based on the measurement of the decrease in length of threads of coagulated egg albumin enclosed in short lengths of glass tubing during twenty-four hours' digestion, and also by Grützner's method (Pfluger's *Arch.*, **144**, 545), in which fibrin stained with carmine is digested for a short time, and the amount dissolved estimated from the colour of the liquid. The latter method is preferable where a rapid estimation is desired or where the peptic activity of the juice investigated is small. Pepsin preparations obtained from the mucous membrane of pigs' stomachs by Pekelharing's method (*Zeitsch. physiol. Chem.*, 1896, **22**, 233; **35**, 8) can be used as a standard in these determinations, as they are very uniform in activity and can be kept for a long time without deteriorating.

Estimation of the Acidity of Urine. J. Clarens. (*Compt. rend.*, 1915, **160**, 814-817.)—The alkaline or acid reaction of urine is governed by that of the blood, and the active elements involved may be expressed in terms of sodium carbonate on the one hand and monosodium dihydrogen phosphate on the other. The direct titration of such a system with standard sodium hydroxide in presence of phenolphthaleïn may lead to quite erroneous results, which are influenced by the absorption of carbon dioxide from the air or by the expulsion of carbon dioxide through agitation when liberated by the mutual reaction of the components. A simple method for the estimation of the actual equilibrium of acid and basic constituents of the urine is described: A measured volume of urine is treated with a known quantity of $\frac{N}{10}$ hydrochloric acid, boiled to expel the carbon dioxide, and then immediately cooled; the excess of acid is titrated with $\frac{N}{10}$ sodium hydroxide in presence of phenolphthaleïn. The volume of standard acid is subtracted from the volume of alkali used. If the result is positive, the urine is acid; if negetive, it is alkaline. Thus sodium carbonate and bicarbonate are regarded as equivalent in the basic sense, and such a view is physiologically correct. In carrying out the estimation, the ebullition should not be prolonged so as to cause a loss of volatile acids

or hydrolysis of the urea. The titration should always be performed in the cold on account of the presence of ammonium salts. If the liquid become deeply coloured as the result of boiling, it should be diluted with recently boiled water before titration.

J. F. B.

Estimation of Indican in Urine. A. Jolles. (*Zeitsch. physiol. Chem.*, 1915, **94**, 79-103; through *J. Soc. Chem. Ind.*, 1915, **34**, 851.)—Indoxyl condenses with thymol in presence of ferric chloride and hydrochloric acid to form a compound, $C_{19}H_{17}O_2N$, probably 4-cymene-2-indolindolignone (Friedländer's nomenclature), which forms red crystals melting with decomposition at 218°-220° C., and yields an intensely violet monohydrochloride which is very readily hydrolysed. This reaction can be applied to the detection or estimation of indican in urine. Ten c.c. of the urine are mixed with 1 c.c. of a 5 per cent. solution of thymol in alcohol, and 10 c.c. of fuming hydrochloric acid containing 5 grms. of ferric chloride per litre, and after fifteen minutes the mixture is extracted with 4 c.c. of chloroform, which is coloured violet if the urine contained as little as 0·0032 mgrm. of indican. For quantitative purposes, the urine is treated with one-tenth of its volume of basic lead acetate solution and filtered; 5 to 10 c.c. of the filtrate are mixed with the thymol and ferric chloride solutions in the same proportions as for the qualitative test, and after standing for two hours the liquid is extracted with portions of 5 c.c. of chloroform so long as the extracts are coloured. The united extracts are washed once with water, whereupon the colour changes to reddish-brown owing to hydrolysis of the hydrochloride, once with very dilute alkali ($\frac{N}{500}$ to $\frac{N}{1000}$), and once again with water. Any colouring matter in the first water washing is extracted by a few c.c. of chloroform, which is then washed with alkali with the rest. The total extract is made up to 25 or 50 c.c. with chloroform and compared colorimetrically with a suitably diluted standard solution containing 0·01 grm. of the colouring matter (corresponding to 0·009 grm. of indican) in 100 c.c. of chloroform.